Molecular characterization of the human NANOG protein.

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Public Summary:
NANOG is a key transcriptional regulator of pluripotent stem cell (PSC) self-renewal. NANOG occupies promoters that are active and others that are repressed during self-renewal; however, the mechanisms by which NANOG regulates transcriptional repression and activation are unknown. We hypothesized that individual protein domains of NANOG control its interactions with both the promoters and its coregulators. We performed a detailed characterization of the functional domains in the human (h) NANOG protein, using a panel of deletion-mutant and point-mutant constructs. We determined that six amino acids in the homeodomain ((136)YKQVKT(141)) are sufficient for the nuclear localization of hNANOG. We also determined that the tryptophan-rich region (W) of hNANOG contains a CRM1-independent signal for nuclear export, suggesting a possible cellular shuttling behavior that has not been reported for hNANOG. We also show that at least four tryptophans are required for nuclear export. We also determined that similar to murine (m) NANOG, the W region of hNANOG contains a homodimerization domain. Finally, in vitro transactivation analyses identified distinct regions that enhance or diminish activity at gene promoters that are active during self-renewal. Specifically, the N-terminal region interferes with transcription and removal of this region that produced a "super-active" hNANOG with enhanced transcriptional activity. We also confirmed that the transcriptional activator in hNANOG is contained in the C-terminal region, similar to murine NANOG. In summary, this study has characterized the structure and function of hNANOG protein leading to an increased understanding of the mechanism by which hNANOG regulates both transcriptional activation and repression during PSC self-renewal.

Scientific Abstract:
NANOG is a key transcriptional regulator of pluripotent stem cell (PSC) self-renewal. NANOG occupies promoters that are active and others that are repressed during self-renewal; however, the mechanisms by which NANOG regulates transcriptional repression and activation are unknown. We hypothesized that individual protein domains of NANOG control its interactions with both the promoters and its coregulators. We performed a detailed characterization of the functional domains in the human (h) NANOG protein, using a panel of deletion-mutant and point-mutant constructs. We determined that six amino acids in the homeodomain ((136)YKQVKT(141)) are sufficient for the nuclear localization of hNANOG. We also determined that the tryptophan-rich region (W) of hNANOG contains a CRM1-independent signal for nuclear export, suggesting a possible cellular shuttling behavior that has not been reported for hNANOG. We also show that at least four tryptophans are required for nuclear export. We also determined that similar to murine (m) NANOG, the W region of hNANOG contains a homodimerization domain. Finally, in vitro transactivation analyses identified distinct regions that enhance or diminish activity at gene promoters that are active during self-renewal. Specifically, the N-terminal region interferes with transcription and removal of this region that produced a "super-active" hNANOG with enhanced transcriptional activity. We also confirmed that the transcriptional activator in hNANOG is contained in the C-terminal region, similar to murine NANOG. In summary, this study has characterized the structure and function of hNANOG protein leading to an increased understanding of the mechanism by which hNANOG regulates both transcriptional activation and repression during PSC self-renewal.